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Tracking bone marrow stromal cells with iron labelling

E. Farrell¹, P. Wielopolsky², H. Jahr³, J. Verhaar³, H. Weinans³, G. Krestin², F. O'Brien⁴, M. Bernsen², G. van Osch⁵;

¹Center For Bioengineering, Trinity College Dublin & Royal College of Surgeons in Ireland, Dublin, Ireland, ²Radiology, Erasmus MC, Rotterdam, Netherlands, ³Orthopaedics, Erasmus MC, Rotterdam, Netherlands, ⁴X, Royal College of Surgeons, Dublin, Ireland, ⁵Orthopaedics & Otorhinolaryngology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands

Purpose: The use of bone marrow stromal cells (BMSCs) transplantation for cartilage repair is receiving increasing attention. Knowledge about the fate of these cells following implantation is of importance. Recently, the ability to label cells with iron oxide particles and visualise them using MRI has been demonstrated. The aim of this study was to examine the ability to label human BMSCs with superparamagnetic iron oxide (SPIO) and to assess the effect on differentiation potential and long term fate in-vivo following seeding into scaffolds.

Methods and Materials: Adult human BMSCs were labelled with SPIO and cultured in osteogenic, chondrogenic, adipogenic or control medium for 13 days. Cells were harvested for histology, MRI and RT-PCR. Cells were also seeded onto collagen-glycosaminoglycan scaffolds for 21 days and then implanted subcutaneously into nude mice for further 28 days.

Results: MRI demonstrated iron taken up by cells and retained for 13 days, during which time BMSCs differentiated along different lineages. Osteogenesis, chondrogenesis and adipogenesis were demonstrated in SPIO-labelled cells by histology. While there was no apparent difference in the ability to differentiate at the histological level, subtle but sometimes significant differences when found by PCR. Expression of chondrogenic markers can be increased by SPIO-labelling and in the in-vivo experiment increased cartilage formation was visible. The potential of this method was demonstrated by tracking labelled cells in vivo by MRI seven weeks after labelling.

Conclusions: BMSCs can be labelled with SPIO and tracked by MRI. Labelling seems to stimulate chondrogenesis.

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Subtractive gene expression profiling of articular cartilage and mesenchymal stem cells: Serpins as cartilage-relevant differentiation markers.

S. Boeuf¹, E. Steck², K. Pelttari¹, T. Hennig¹, A. Buness³, K. Benz⁴, D. Witte¹, H. Sültmann³, A. Poustka³, W. Richter¹;

¹Division Of Experimental Orthopaedics, Orthopaedic Clinic, University of Heidelberg, heidelberg, Germany, ²Division Of Experimental Orthopaedics, Orthopaedic University Hospital Heidelberg, Heidelberg, Germany, ³Division Of Molecular Genome Analysis, German Cancer Research Center, heidelberg, Germany, ⁴Cell Biology, NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany

Purpose: Mesenchymal stem cells (MSC) are a population of cells broadly discussed to support cartilage regeneration. The differentiation of MSC into articular chondrocytes is, however, still poorly understood on the molecular level. The aim of this study was to perform an almost genome-wide screen for genes differentially expressed between cartilage and MSC and to extract new markers useful to define chondrocyte differentiation stages.

Methods and Materials: Gene expression profiles of MSC (n=8) and articular cartilage (n=7) were compared on a 30,000 cDNA-fragment array and differentially expressed genes were extracted by subtraction. Expression of selected genes was assessed during in vitro chondrogenic differentiation of MSC and during dedifferentiation of expanded chondrocytes using quantitative and semi-quantitative RT-PCR. Protein secretion was measured by ELISA.

Results: Eighty-seven genes were differentially expressed between MSC and cartilage with a more than 3-fold difference. Sixty-seven of them were higher expressed in cartilage and among them 31 genes were previously not detected in cartilage. Differential expression was confirmed for 69% of 26 reanalysed genes by RT-PCR. The profiles of 3 unknown transcripts and 6 protease-related molecules were characterised during differentiation. SERPINA1 and SERPINA3 mRNA expression correlated with chondrogenic differentiation of MSC and dedifferentiation of chondrocytes, and SERPINA1 protein levels in culture supernatants could be correlated alike.

Conclusions: cDNA-array analysis identified SERPINA1 and A3 as new differentiation-relevant genes for cartilage. Since SERPINA1 secretion correlated with both chondrogenesis of MSC and dedifferentiation during chondrocyte expansion, it represents an attractive marker for refinement of chondrocyte differentiation.

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The effect of medium osmolality on in situ chondrocyte death within wounded articular cartilage

A.K. Amin¹, J.S. Huntley¹, P.G. Bush², A.H. Simpson³, A.C. Hall⁴;

¹Orthopaedic And Trauma Surgery, Royal Infirmary of Edinburgh/ University of Edinburgh, Edinburgh, United Kingdom, ²Biomedical And Laboratory Sciences, University of Edinburgh, Edinburgh, United Kingdom, ³Orthopaedic And Trauma Surgery, University of Edinburgh, Edinburgh, United Kingdom, ⁴School Of Biomedical And Clinical Laboratory Sciences, University of Edinburgh, Edinburgh, United Kingdom

Purpose: Determine whether medium osmolality affects in situ chondrocyte death at a scalpel wounded cartilage edge.

Methods and Materials: Osteochondral explants (n=48) harvested from bovine metacarpophalangeal joints were exposed to distilled water (0 mOsm), 170 mOsm Dulbecco's Modified Eagle's Medium (DMEM), 340 mOsm DMEM (control) or 480 mOsm DMEM for 90 seconds before the full thickness of articular cartilage was wounded with a scalpel. Explants were incubated in the same media for 2.5 hours and transferred to 340 mOsm DMEM for a 7-day culture at 37°C. The spatial distribution of chondrocyte death and percentage cell death at the wounded cartilage edge were compared as a function of medium osmolality and time (2.5 hours vs. 7 days) using confocal laser scanning microscopy.

Results: Chondrocyte death was localised within the superficial zone of articular cartilage. Compared to the control explants exposed to 340 mOsm, percentage cell death was highest for explants exposed to distilled water, similar for explants exposed to 170 mOsm and lowest for explants exposed to 480 mOsm at 2.5 hours (13.0% (control), 35.5% (p=0.02), 10.4% (p=0.9) and 4.3% (p=0.01) respectively) and 7 days (9.9 % (control), 37.7% (p=0.004), 13.9% (p=0.2) and 3.5% (p=0.01) respectively). There was no significant change in percentage cell death from 2.5 hours to 7 days at any medium osmolality.

Conclusions: Medium osmolality significantly influences chondrocyte death in wounded cartilage (inverse relationship). Cell death occurs within 2.5 hours with no progression upto 7 days. These data have important clinical relevance for the osmolality of joint irrigating solutions used during osteoarticular surgery.

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Full thickness cartilage lesion did not affect knee function preoperatively in ACL injured subjects

V. Hjermundrud¹, T. Kvist², M.A. Risberg³, L. Engebretsen⁴, A. Årøen⁵;

¹Department Of Physiotherapy, Akershus University Hospital, Lørenskog, Norway, ²University Of Oslo, Ullevål University Hospital, Oslo, Norway, ³Nar, Ullevål University Hospital, Oslo, Norway, ⁴Orthopaedic Centre, Ullevål University Hospital, Oslo, Norway, ⁵Orthopaedic Center, Ullevål University Hospital, University of Oslo, Oslo, Norway

Purpose: The objective of this study was to investigate differences in preoperative knee function in ACL injured subjects with or without full thickness cartilage defects (ICRS grade 3 or 4).

Methods and Materials: A cross sectional study using data from the Norwegian National ACL Register (3704 primary ACL-surgery cases) was conducted. Thirty five patients met the following inclusion criteria: less than one year since knee injury, no meniscus injury, a full thickness chondral lesion of more than 2 cm² and age less than 40 years old. The control group (n=970) was comparable with the same characteristics, but no cartilage lesion was noted. All patients completed preoperatively a functional scoring form; the Knee Osteoarthritis Outcome score (KOOS) consisting of five subscales and the surgeon preoperatively recorded the depth of the cartilage lesion according to the ICRS scale and the size of the lesion.

Results: No significant difference for the ACL patients with no meniscus injury was found for none of the five subgroups in KOOS-score. A chondral lesion was located on the medial femoral condyle in 62 % of the 35 patients with cartilage lesion. There was a significant higher mean age (29 years versus 23 in the control group) and predominantly males (68 %) in the cartilage lesion group compared to the control group (54 %).

Conclusions: The combination of a full thickness cartilage lesion of more than 2 cm² did not result in an inferior knee function before ACL-reconstruction evaluated using the KOOS functional outcome scores.